Natural Limits of Pregnancy Testing in Relation to the Expected Menstrual Period

Allen J. Wilcox, MD, PhD

Donna Day Baird, PhD

David Dunson, PhD

Ruth McChesney, PhD

Clarice R. Weinberg, PhD

THE PRACTICAL REFERENCE POINT for the detection of pregnancy is the first day of a woman's missed period, ie, the day on which she expects her next period to begin. Unless a woman carries out special tests for ovulation, her only basis for predicting the onset of her next menses is her usual cycle length. Most women are able to report their usual cycle length, despite the natural variability from cycle to cycle. This variability in cycle length is due largely to variation in the number of days from the beginning of menses to ovulation.[1]

There is no practical way to identify conception before implantation of the blastocyst. The time of implantation has its own natural variability, ranging from 6 to 12 days after ovulation.[2] Thus, the exact interval from onset of previous menstrual period to ovulation and then to the detectability of a pregnancy is inherently difficult to predict.

The trophoblast cells of the conceptus produce human chorionic gonadotropin (hCG) in amounts that increase exponentially following implantation. This production of hCG provides the basis for all pregnancy tests, including home test kits. In 1999, about 19 million over-the-counter pregnancy test kits were sold in the United States, with sales of about $230 million.[3] Kits typically instruct women to test for pregnancy "as

See also Patient Page.

Context Pregnancy test kits routinely recommend testing "as early as the first day of the missed period." However, a pregnancy cannot be detected before the blastocyst implants. Due to natural variability in the timing of ovulation, implantation does not necessarily occur before the expected onset of next menses.

Objective To estimate the maximum screening sensitivity of pregnancy tests when used on the first day of the expected period, taking into account the natural variability of ovulation and implantation.

Design and Setting Community-based prospective cohort study conducted in North Carolina between 1982 and 1986.

Participants Two hundred twenty-one healthy women 21 to 42 years of age who were planning to conceive.

Main Outcome Measures Day of implantation, defined by the serial assay of first morning urine samples using an extremely sensitive immunoradiometric assay for human chorionic gonadotropin (hCG), relative to the first day of the missed period, defined as the day on which women expected their next menses to begin, based on self-reported usual cycle length.

Results Data were available for 136 clinical pregnancies conceived during the study, 14 (10%) of which had not yet implanted by the first day of the missed period. The highest possible screening sensitivity for an hCG-based pregnancy test therefore is estimated to be 90% (95% confidence interval [CI], 84%-94%) on the first day of the missed period. By 1 week after the first day of the missed period, the highest possible screening sensitivity is estimated to be 97% (95% CI, 94%-99%).

Conclusions In this study, using an extremely sensitive assay for hCG, 10% of clinical pregnancies were undetectable on the first day of missed menses. In practice, an even larger percentage of clinical pregnancies may be undetected by current test kits on this day, given their reported assay properties and other practical limitations.

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early as the first day of the missed period." To evaluate the number of pregnancies detectable at the missed period, we estimated the day of implantation relative to the expected first day of the missed period among a group of women with naturally conceived pregnancies.

METHODS

We studied 221 healthy North Carolina women who were planning to become pregnant.[4] Women with no known fertility problems were recruited in 1982-1986 from the local community and enrolled at the time

they discontinued their method of birth control. Women ranged from 21 to 42 years of age; mean age was 30 years, with 5% older than 35 years. Most were college educated, and 96% were white; further details are provided elsewhere.[4] All participants provided informed consent, and the study was ap-

Author Affiliations: Epidemiology Branch (Drs Wilcox and Baird) and Biostatistics Branch (Drs Dunson and Weinberg), National Institute of Environmental Health Sciences, Durham, NC; and Department of Biological Sciences, Barnard College, Columbia University, New York, NY (Dr McChesney).
Corresponding Author and Reprints: Allen J. Wilcox, MD, PhD, Epidemiology Branch, MD A3-05, National Institute of Environmental Health Sciences, Durham, NC 27709 (e-mail: wilcox@niehs.nih.gov).

Table. Estimated Day of Implantation of Clinical Pregnancies Relative to the Expected Onset of the Next Menstrual Period

Estimated Day of Implantation Relative to First Day of the Expected Period	Estimated No. of Conceptions Implanting on This Day	Cumulative %
–7 and earlier	55	40
–6	15	51
–5	14	62
–4	9	68
–3	7	74
–2	8	79
–1	8	85
Day of expected period	6	90
+1	1	90
+2	3	93
+3	3	95
+4	2	96
+5	1	97
+6	0	97
+7	0	97
+8	1	98
+9	0	98
+10	1	99
+11 and later	2	100
Total	136	

proved by the National Institute of Environmental Health Sciences institutional review board. Women kept menstrual diaries and froze daily urine specimens for later analysis. During the study, 151 women conceived a clinical pregnancy (defined as a pregnancy lasting 6 weeks beyond the last menstrual period).

Urine samples were assayed for hCG with an extremely sensitive immunoradiometric assay.[5] Detection limit was 0.01 ng of hCG per milliliter, which is approximately 0.13 mIU/mL.[4] This assay was directed primarily at intact hCG, but also cross-reacted with the free-beta subunit of hCG. The hCG produced by clinical pregnancy was easily distinguished from background levels by its pattern of exponential rise. We defined the onset of pregnancy-related hCG as the earliest day of sustained hCG elevation in which hCG concentration exceeded 0.015 ng/mL. We regard this as the best available marker of implantation.[2]

Among the 151 clinical pregnancies, we were unable to determine the onset of hCG for 9 (in most cases because crucial urine samples were missing). Of the remaining 142 preg-

nant women, 136 provided information about their "usual cycle length." These 136 women provided the basis for the current analysis. The median reported cycle length was 29 days, with 96% of women reporting lengths between 23 and 35 days (range, 19-60 days). We previously tested the validity of self-reported cycle length by comparing it with the observed length of the woman's follicular phase during the study. The correlation was 0.55.[6]

Ninety-four of the eligible 136 women reported their usual cycle length as a single number. We used this usual cycle length to estimate the day on which a woman would expect her next period (eg, a woman with a usual cycle length of 28 days would expect her period on day 29.) For women who conceive, the day on which the next period is expected is the "first day of the missed period." The remaining 42 women reported their usual cycle length as a range (eg, "28-30 days"). For these women, we used the upper limit of the range to determine the first day of the missed period.

RESULTS

The TABLE shows the estimated day of implantation relative to the first day of the missed period. Implantation occurred by the first day of the missed period in 90% of pregnancies (95% confidence interval [CI], 84%-94%). By 7 days after the first day of the missed period, 97% of all clinical pregnancies had implanted (95% CI, 94%-99%). After excluding the 21 women who reported that their cycles were "irregular," results were unchanged (90% and 97%).

COMMENT

Implantation can occur surprisingly late in relation to a woman's expected menses. For 10% of clinical pregnancies in our study, implantation occurred after the first day of the next expected period. This represents an insurmountable limitation of hCG-based pregnancy testing on the first day of the missed period. A perfectly sensitive assay for hCG could not have detected 100% of these clinical preg-

nancies even 10 days after the period was expected (Table).

These data describe the physiologic limits of early pregnancy testing. However, we did not directly test the performance of home test kits. The performance of a single, qualitative assay in the hands of a layperson depends on many factors. One factor is the assay detection limit. While detection limits are not routinely provided with test kits, a Web site lists assay detection limits for 40 commercial kits as determined by telephoning the manufacturers.[7] These reported assay detection limits of hCG range from 15 to 100 mIU/mL, with most kits between 25 and 50 mIU/mL. Detection limits for some kits may be slightly more sensitive than reported by manufacturers.[8] Even so, an assay detection limit of 15 mIU/mL is about 100 times less sensitive than the assay we used to define implantation (0.13 mIU/mL). This implies that most current test kits would not reliably detect hCG on the day of implantation. Chard[9] estimates that an assay with a detection limit of 25 mIU/mL will begin to detect pregnancy around 3 or 4 days after implantation.

Other factors also affect the performance of test kits. Urinary dilution may reduce detection limits.[10] Not all test kits measure the same components of hCG; some measure only intact hCG, while others measure intact plus the free beta.[11,12] The ratio of these hCG components may vary from pregnancy to pregnancy,[11] which could affect the detection limits of specific tests. In addition, user errors have been reported to contribute to false-negative findings.[13]

The instruction to test on the "first day of the missed period" may not mean the same thing to all women. Women do not know their period is late until the second day of their expected period. If women think the first day of their missed period is after they know their period is late, the percent of false-negative test results would decrease slightly.

How all these factors affect actual test kit performance is hard to predict. If kits do reliably detect pregnancy by the third

day after implantation, then about one fourth of clinical pregnancies would produce a false-negative test result on the first day of the missed period. Previous studies do not provide information on this point because they lacked proper attention to the timing of the test in relation to expected menses.[13]

It should not necessarily be the goal of home test kits to acheive the lowest possible limits of hCG detection. About one fourth of all pregnancies detectable at implantation fail very early.[4] The detection of these events by highly sensitive home test kits would be of uncertain benefit to women.

The interpretation of a negative pregnancy test result on the first day of the missed period deserves comment. Some package inserts state that if a test is nega-tive, "you are probably not pregnant." One says "you are NOT PREGNANT" (emphasis in the original). This unfounded assurance could have important consequences. For example, women with a negative test result may fail to protect themselves from exposures to toxicants in the workplace or to medications that could damage a developing embryo.

In summary, the timing of implantation varies widely in its relation to the expected period. Many women will test positive a week or more before their period is expected, while a few women will test positive only a week or more afterward. Adolescents and young women are frequent users of test kits[14] but may be especially prone to false-positive test results because they are at

high risk for delayed ovulation.[15] Better, information on the limits of early testing can help balance the costs and benefits of early detection against the risks of a false-negative test result.

REFERENCES

1. Lenton EA, Landgren B, Sexton L, et al. Normal variation in the length of the follicular phase of the menstrual cycle: effect of chronological age. *Br J Obstet Gynaecol.* 1984;91:681-684.
2. Wilcox AJ, Baird DD, Weinberg CR. Time of implantation of the conceptus and loss of pregnancy. *N Engl J Med.* 1999;340:1796-1799.
3. Lipsitz R. Pregnancy tests. *Sci Am.* 2000;283:110-111.
4. Wilcox AJ, Weinberg CR, O'Connor JF, et al. Incidence of early loss of pregnancy. *N Engl J Med.* 1988;319:189-194.
5. Armstrong EG, Ehrlich PH, Birken S, et al. Use of a highly sensitive and specific immunoradiometric assay for detection of human chorionic gonadotropin in urine of normal, nonpregnant, and pregnant individuals. *J Clin Endocrinol Metab.* 1984;59:867-874.
6. Wilcox AJ, Dunson D, Baird DD. The timing of the fertile window in the menstrual cycle: day-specific estimates from a prospective study. *BMJ.* 2000;321:1259-1262.
7. Fertility Plus. Available at: http://www.fertilityplus.org/faq/htp.htm. Accessibility verified September 6, 2001.
8. Mishalani SH, Seliktar J, Braunstein GD. Four rapid serum-urine combination assays of choriogonadotropin (hCG) compared and assessed for their utility in quantitative determinations of hCG. *Clin Chem.* 1994;40:1944-1949.
9. Chard T. Pregnancy tests: a review. *Hum Reprod.* 1992;7:701-710.
10. Ikomi A, Matthews M, Kuan AM, et al. The effect of physiological urine dilution on pregnancy test results in complicated early pregnancies. *Br J Obstet Gynaecol.* 1998;105:462-465.
11. Cole LA, Seifer DB, Kardana A, et al. Selecting human chorionic gonadotropin immunoassays: consideration of cross-reacting molecules in first-trimester pregnancy serum and urine. *Am J Obstet Gynecol.* 1993;168:1580-1586.
12. Alfthan H, Bjoerses U-M, Tiitinen A, et al. Specificity and detection limit of ten pregnancy tests. *Scand J Clin Lab Invest.* 1993;53(suppl 216):105-113.
13. Bastian LA, Nanda K, Hasselblad V, et al. Diagnostic efficiency of home pregnancy test kits: a meta-analysis. *Arch Fam Med.* 1998;7:465-469.
14. Shew ML, Hellerstedt WL, Sieving RE, Smith AE, Fee RM. Prevalence of home pregnancy testing among adolescents. *Am J Public Health.* 2000;90:974-976.
15. Harlow SD, Lin X, Ho JM. Analysis of menstrual diary data across the reproductive life span: applicability of the bipartite model approach and the importance of within-woman variance. *J Clin Epidemiol.* 2000;53:722-733.

Lee Goldman, MD, MPH
Pamela G. Coxson, PhD
Department of Medicine
University of California, San Francisco

Elizabeth Ross, MD
Irwin Rosenberg, MD
Division of Clinical Nutrition
Tufts University
Boston, Mass

Milton C. Weinstein, PhD
M. G. Myriam Hunink, MD, PhD
Paula A. Goldman, MPH
Lawrence Williams, MS
Department of Health Policy and Management
Harvard School of Public Health
Boston

1. Bostom AG, Liaugaudas G, Jacques PF, Rosenberg IH, Selhub J. Total homocysteine lowering treatment among coronary artery disease patients in the era of folic acid fortified grain cereal grain flour [abstract]. *Circulation*. 2001;103:1367.
2. Title LM, Cummings PM, Giddens K, Genest JJ Jr., Nassar BA. Effect of folic acid and antioxidant vitamins on endothelial dysfunction in patients with coronary artery disease. *J Am Coll Cardiol*. 2000;36:758-765.
3. Homocysteine Lowering Trialists' Collaboration. Lowering blood homocysteine with folic acid based supplements: meta-analysis of randomised trials. *BMJ*. 1998;316:894-898.
4. Hunink MG, Goldman L, Tosteson AN, et al. The recent decline in mortality from coronary heart disease, 1980-1990: the effect of secular trends in risk factors and treatment. *JAMA*. 1997;277:535-542.
5. Schnyder G, Roffi M, Pin R, et al. Decreased rate of coronary restenosis after lowering of plasma homocysteine levels. *N Engl J Med*. 2001;345:1593-1600.

CORRECTIONS

Incorrect Wording: The Brief Report entitled "Natural Limits of Pregnancy Testing in Relation to the Expected Menstrual Period" published in the October 10, 2001, issue of THE JOURNAL (2001;286:1759-1761) contained incorrect wording. On page 1761, last paragraph, the third sentence should be: "Adolescents and young women are frequent users of test kits[14] but may be especially prone to false-negative test results because they are at high risk for delayed ovulation.[15]"

Incorrect Wording and Omissions: In the Caring for the Critically Ill Patient article titled "High-Dose Antithrombin III in Severe Sepsis: A Randomized Controlled Trial" published in the October 17, 2001, issue of THE JOURNAL (2001;286:1869-1878), several errors were printed. On page 1874 in the first column, second paragraph, the sentence should read "Antithrombin III resulted in a 15% relative improvement in 90-day mortality," not "absolute improvement." On page 1875, the title of Table 3 should read "Incidence of New Organ Dysfunction According to Logistic Organ Dysfunction Score. . . . " Also on page 1875, the legend for Figure 5 should indicate that the patients represented in the graph are the percentage discharged alive from ICU in relation to all patients (surviving and dead). On page 1876, the footnote to Table 4 was inadvertently omitted. It should have read "Number of patients actually treated with antithrombin III or placebo." This footnote clarifies the different patient numbers for the primary efficacy analysis (n=1157 per group) vs the safety analysis (placebo: n=1155; antithrombin III: n=1161). On page 1876, the top paragraph in the third column should end with this sentence: "Up to and beyond the second interim analysis, an overall nominal survival benefit for antithrombin III prevailed, despite the recognized increased bleeding risk in that group."

Incorrect Date: In the Original Contribution entitled "Survival by Time of Day of Hemodialysis in an Elderly Cohort" published in the December 5, 2001, issue of THE JOURNAL (2001;286:2690-2694), there was an incorrect date in the abstract under "Design." The year after the word "baseline" should be 1988, not 1998.